

ABSTRACT

A method is provided for producing motif-specific, context-independent antibodies which recognize a plurality of peptides or proteins within a genome that contain the same motif. The method includes the steps of (1) constructing a degenerate peptide library featuring (i) a fixed target motif containing one or more invariant amino acids including at least one modified amino acid, and (ii) a plurality of degenerate amino acids flanking the motif, (2) immunizing a host with this peptide library to raise a context-independent antibody against all or part of the target motif, and (3) isolating antisera from the host and purifying the motif-specific, context-independent antibody from the antisera, the antibody recognizing a plurality of peptides or proteins within a genome that contain the motif. Motif-specific, context-independent antibodies produced by the disclosed method are also provided. The disclosed method encompasses motifs consisting of a single modified amino acid, such as phosphothreonine, phosphoserine, phosphotyrosine, acetyl-lysine, and nitrotyrosine, as well as short recurring motifs comprising multiple invariant amino acids, such as all or part of MAPK consensus substrate motifs, CDK consensus substrate motifs, PKA consensus substrate motifs, Akt consensus substrate motifs, PKC consensus substrate motifs, ATM consensus substrate motifs, 14-3-3 consensus binding motifs, PDK1 consensus docking motifs, phosphothreonine-X-(arginine(R)/lysine(K)), PKC Zeta consensus substrate motifs, ABL kinase consensus substrate motifs, CDK5 consensus substrate motifs, insulin receptor consensus substrate motifs, PI3K P85 consensus binding motifs, CaMKII consensus substrate motifs, SRC kinase consensus substrate motifs, CDC2/CDK2 consensus substrate motifs, GSK3 kinase consensus substrate motifs, and proline(P)-(phosphoserine/phosphothreonine)-proline(P). The antibodies of the invention are not limited, however, to these exemplary motifs. Methods of using the antibodies, *e.g.* for genome-wide profiling, are also provided.